In the Specification:

On page 18, amend the description of Figure 3 as follows:

C1

FIGURE 3. Unique Overhangs and Unique Couplings. Figure 3 illustrates the point that the number of unique overhangs of each size (e.g. the total number of unique overhangs composed of 1 or 2 or 3, etc. nucleotides) exceeds the number of unique couplings that can result from the use of all the unique overhangs of that size. For example, there are 4 unique 3' overhangs composed of a single nucleotide, and 4 unique 5' overhangs composed of a single nucleotide. Yet the total number of unique couplings that can be made using all the 8 unique single-nucleotide 3' overhangs and single-nucleotide 5' overhangs is 4[.], as presented in Panel A. Panel B shows that the number of unique single-nucleotide 3' overhangs is greater than the number of unique couplings. Thus, only 2 intrinsically unique couplings exist using single-nucleotide 3' overhangs as shown. Panel C shows 4 unique-single nucleotide 5' overhangs are possible (i.e., A, C, G, & T). For each of these there is a complementary 5' overhang with which it can pair (i.e., T, G, C, & A, respectively), as shown. Panel D shows that the number of unique single-nucleotide 5' overhangs is greater than the number of unique couplings. Thus, only 2 intrinsically unique couplings exist using single-nucleotide 5' overhangs as shown.

On page 19, please amend the description of Figure 4 as follows:

C2

FIGURES 4A and 4B. Unique Overall Assembly Order Achieved by Sequentially Coupling the Building Blocks. Awareness of the degeneracy (between the number of unique overhangs and the number of unique couplings) is important in order to avoid the production of degeneracy in the overall assembly order of the finalized nucleic acid.

However, a unique overall assembly order can also be achieved – despite the use of non-unique couplings – by using building blocks having distinct combinations of couplings, and/or by stepping the assembly of the building blocks in a deliberately chosen sequence.

In Figure 4A, Panel A, for example, one could attempt to assemble the following nucleic acid product using the 5 nucleic acid building blocks as shown. Figure 4A illustrates the fact that in order to assemble a total of "n" nucleic acid building blocks, "n-1" couplings are needed. Yet it is sometimes the case that the number of unique couplings available for use is fewer than the "n-1" value. In Figure 4A, Panel B, degeneracy in the overall assembly order of the 5 nucleic acid building blocks would be present if the assembly process were carried out in one step. For example, building block #2 and building block #3 could both couple to building block #1 as shown. Under these, and other, circumstances a stringent non-stochastic overall assembly order can still be achieved by performing the assembly process in sequential steps.

C2 cont

For example, Figure 4B illustrates a unique overall assembly order could be achieved by sequentially coupling the building blocks in 2 steps (rather than all at once) as shown. In this example, 2 sequential steps are used to achieve a designed overall assembly order for five nucleic acid building blocks. In this illustration the designed overall assembly order for the five nucleic acid building blocks is: 5'-(#1-#2-#3-#4-#5)-3', where #1 represents building block number 1, etc.

On page 19, please amend the description of Figure 5 as follows:

FIGURE 5. Unique Couplings Available Using a Two-Nucleotide 3' Overhang.

C3

Figure 5 further illustrates the point that the number of unique overhangs of each size

(here, e.g. the total number of unique overhangs composed of 2 nucleotides) exceeds the number of unique couplings that can result from the use of all the unique overhangs of that size. For example, there are 16 unique 3' overhangs composed of two nucleotides, and another 16 unique 5' overhangs composed of two nucleotides, for a total of 32 as shown. Yet the total number of couplings that are unique and not self-binding that can be made using all the 32 unique double-nucleotide 3' overhangs and double-nucleotide 5' overhangs is 12. Some apparently unique couplings have "identical twins" (marked in the same shading), which are visually obvious in this illustration. Still other overhangs contain nucleotide sequences that can self-bind in a palindromic fashion, as shown and labeled in this figure; thus they do not contribute the high stringency to the overall assembly order.

C3 cont.

On page 21, please amend the description of Figure 9 as follows:

FIGURE 9. Addition of Introns by Synthetic Ligation Reassembly. Figure 9 shows in generic cartoon form that an intron may be introduced into a chimeric progeny molecule by way of a nucleic acid building block. It is appreciated that introns often have consensus sequences at both termini in order to render them operational. It is also appreciated that, in addition to enabling gene splicing, introns may serve an additional purpose by providing sites of homology to other nucleic acids to enable homologous recombination. For this purpose, and potentially others, it may be sometimes desirable to generate a large nucleic acid building block for introducing an intron. If the size is overly large easily [genrating] generated by direct chemical synthesis of two single stranded oligos, such a specialized nucleic acid building block may also be generated by direct

C4

C4 cont...

chemical synthesis of more than two single stranded oligos or by using a polymerase-based amplification reaction as shown in Figure 2.

On page 21, please amend the description of Figure 10 as follows:

C5

FIGURE 10. Ligation Reassembly Using Fewer Than All the Nucleotides of an Overhang. Figure 10 shows that coupling can occur in a manner that does not make use of every nucleotide in a participating overhang. The coupling is particularly [lively] <u>likely</u> to survive (e.g. in a transformed host) if the coupling reinforced by treatment with a ligase enzyme to form what may be referred to as a "gap ligation" or a "gapped ligation." It is appreciated that, as shown, this type of coupling can contribute to generation of unwanted background product(s), but it can also be used advantageously to increase the diversity of the progeny library generated by the designed ligation reassembly. <u>The example in Figure 10 shows ligation of one strand only; the gap in the second strand can be repaired in vivo.</u>

On pages 21-22, please amend the description of Figure 11 as follows:

C6

FIGURE 11. Avoidance of unwanted self-ligation in palindromic couplings. As mentioned before and shown in Figure 5, certain overhangs are able to undergo self-coupling to form a palindromic coupling. A coupling is strengthened substantially if it is reinforced by treatment with a ligase enzyme. Accordingly, it is appreciated that the lack of 5' phosphates on these overhangs, a shown, can be used advantageously to prevent this type of palindromic self-ligation. Accordingly, this invention provides that nucleic acid building blocks can be chemically made (or ordered) that lack a 5' phosphate group (or alternatively they can be remove<u>d</u> – e.g. by treatment with a phosphatase enzyme such as

C6 Cont...

a calf intestinal alkaline phosphatase (CIAP) – in order to prevent palindromic self-ligations in ligation reassembly processes. This figure shows that there is no self-ligation of end primers with palindromic overhangs.

On pages 23, at line 24, please amend the description of Figure 17 as follows:

C7

FIGURE 17: Solid Phase Ligation Reassembly. The outline of the procedure used is the following: (1) Annealing of complementary oligos. (Couplings are shown underlined and in bold. Dashes "------" indicate internal sequences not involved in couplings.) (2) Immobilization of 5' pre-annealed biotinylated fragment to conjugated beads. (3) Wash to remove free fragments. (4) Enzymatic ligation of consecutive pre-annealed fragments including washes between each addition to remove free fragments. (5) BsaI-mediated elution of reassembled gene (cuts inside of TOPO sequence). (6) Ligation to 5'- and 3' end PCR generated fragments (if necessary). (7) Cloning into appropriate vector.

On pages 23-24, please amend the description to Figure 18 as follows:

C8

FIGURE 18. Polynucleotide reassembly. Shown is an example of directed evolution. N different strains of a virus are used in this illustration, but the technique is applicable to any single nucleic acid as well as to any nucleic acid for which different strains, species, or gene families have homologous nucleic acids that have one or more nucleotide changes compared to other homologous nucleic acids that have one or more nucleotide changes compared to other homologous nucleic acids. The different variant nucleic acids are experimentally generated, preferably non-stochastically, as described herein, and screened or selected to identify those variants that exhibit the desired property. The

C8 Cont...

directed evolution method(s) and screening can be repeated one or more times to obtain further improvement. Panel B shows that successive rounds of directed evolution can produce progressively enhanced properties, and that the combination of individual beneficial mutations can lead to an enhanced improvement compared to the improvement achieved by an individual beneficial mutation. <u>This figure illustrates the non-stochastic polynucleotide reassembly in combination with non-stochastic polynucleotide site-saturation mutagenesis.</u>

On page 24, please amend the description of Figures 19-A-C as follows:

C9

FIGURES 19A-~~C~~<u>F</u>. An alignment of two CMV-derived nucleotide sequences from human and primate species. Shown here is an alignment of two CMV-derived nucleotide sequences of human and primate strains. This alignment is serviceable for performing non-stochastic polynucleotide reassembly. Nucleotide sequences shared by ~~2~~ <u>two</u> sequences are <u>boxed</u> ~~in red lettering~~ to illustrate preferred but non-limiting examples of reassembly points.

On page 24, please amend the description of Figure 20 as follows:

C10

FIGURE<u>S</u> 20<u>A-B</u>. An alignment of the IFN-gamma nucleotide sequences from human, cat, rodent species. Shown here is an alignment of the IFN-gamma nucleotide sequences from human, cat and rodent species . This alignment is serviceable for performing non-stochastic polynucleotide reassembly. Nucleotide sequences shared by ~~2~~ <u>two or three</u> sequences are <u>boxed</u> ~~in blue lettering & nucleotide sequences shared by 3 sequences are in red lettering~~ to illustrate preferred but non-limiting examples of reassembly points.